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DATA ON SEX DETERMINATION IN CATTLE.[1]

RAYMOND PEARL AND H. M. PARSHLEY.

INTRODUCTION.

In a paper from this Station published over twenty-two years ago Russell[2] presented certain statistics which he collected regarding the relation between the sex of a calf and the time in the period of heat (œstrus) at which the copulation occurred which gave rise to it. Data were collected in regard to (*a*) whether service occurred *early* in the "heat," or in the *middle* of the period of œstrus, or *late* in the "heat"; and (*b*) the sex of the resulting offspring. In the published results only the data for "early" and "late" coitus were presented. The figures given were as follows (*loc. cit.*, p. 209):

"82 cows served during the first part of heat produced 31 bull calves and 51 heifer calves.

"76 cows served during the last part of heat produced 42 bull calves and 34 heifer calves."

These figures were admittedly meager. After the paper referred to was published Dr. Russell continued the collection of statistics on this subject but published nothing further. Upon resigning from the Station staff he very kindly turned over to the senior author of the present paper all his original schedules. The complete collection of statistics has recently been subjected to analysis with the results set forth in this paper.

The original schedules contain spaces for the recording of the following information: (1) Breed and age of bull. (2) Breed of

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 42.

[2] Russell, F. L., "Breeding Statistics," Maine Agr. Expt. Stat., Ann. Rept. for 1891, pp. 208, 209.

cow. (3) Age of cow. (4) Date of service. (5) Date of birth of calf. (6) Sex of calf. (7) Weight of calf. (8) "If you know positively, state whether the cow was served in the first, middle, or last part of heat." Space was provided on each blank for recording a year's breeding operations. These schedules were widely distributed among the leading stock breeders of the state, and the inquiry extended over a period of about four years. As in all such studies a portion of the blanks returned were defective and not usable, either through failure to fill in essential points or from mistakes. One of the commonest defects arose from the fact that the bull was allowed to run in the pasture with the cows, thus precluding any possibility of accurate knowledge of the period of œstrus at which coitus occurred. On the other hand the great majority of the returns were filled up in a very careful and painstaking manner, and furnish valuable data on a number of problems in the physiology of breeding which are much more in the foreground of biological interest at the present time than they were when the inquiry was instituted.

All of the breeds of cattle at all common in the eastern United States are represented in the returns. The majority are either grade or cross-bred animals, but a fair proportion represent "pure bred," registered stock, particularly in the case of the dairy breeds.

In the present paper only a single problem will be discussed. In later communications it is hoped to take up certain other problems in the physiology of breeding for which data are available in these returns. The problem here considered may be stated as follows: Is there any relation and if so of what sort, between the time in period of œstrus at which coitus occurs and the sex of the resulting offspring? This is obviously a matter of much practical as well as theoretical importance. There is perhaps no one thing that would be of more vital importance to the stock-breeder than to be able to control, in some degree even, the sex of the animals which he breeds.

The visible manifestations and the duration of œstrus in the cow are rather variable matters. In the older literature, and in the minds of most practical breeders at the present time, no precise distinction is made between the two divisions of the

œstrous cycle designated by Marshall[1] (p. 36) as the *Proœstrum* and *Œstrus* respectively. The combined period is called "heat" in some cases, whereas in others only a portion of the period of marked sexual activity is so designated. This fact, together with the natural variability of the phenomenon itself amongst different individuals, accounts for the rather widely varying statements found in the literature regarding the duration of "heat" in the cow. Leuckart[2] puts the duration for cow and mare at "about" 4 days. Thury[3] implies a rather shorter duration than this. Flemming[4] says regarding the matter: "The frequency and duration of the period of 'rutting' or 'heat' depends upon the age, species, and other circumstances; but it may be said to persist in the domestic animals from 1 to 15 days at the most. The shortest period is witnessed in the cow and sheep and the longest in the bitch. It is sometimes only present from 12 to 24 hours in some non-fecundated animals." Düsing[5] puts the time of active "heat" in cattle at 24–30 hours at the outside. This is the experience of practical breeders generally.

Under these circumstances it is not to be expected that the returns discussed in this paper are absolutely accurate as to the division of the œstrous period into early, middle, and later portions. No sharp delimiting boundaries between these divisions were, or could be, set in the schedules. The matter had to be left to the judgment of the breeder. We have not been able to discover any reason, however, for supposing the error introduced from the indefiniteness of the phenomenon itself to be biased in one direction or the other. There is unquestionably overlapping of the divisions of the œstrous period in the returns, but there is no reason to suppose that this is not as great proportionately in one direction as in the other. The relation

[1] Marshall, F. H. A., "The Physiology of Reproduction," London, 1910.

[2] Leuckart, R., "Zeugung." In Wagner's "Handwörterbuch der Physiologie," Bd. 4, pp. 707–1101, 1853.

[3] Thury, M., "Ueber das Gesetz der Erzeugung der Geschlechter bei den Pflanzen, den Thieren und dem Menschen," Leipzig, 1864.

[4] Flemming, Geo., "A Text-book of Veterinary Obstetrics," New York, 1879.

[5] Düsing, C., "Die Regulierung des Geschlechtsverhältnisses bei der Vermehrung der Menschen, Tiere und Pflanzen," *Jenaische Zeitschr.*, Bd. 17, pp. 593–940, 1884.

between the returns and the true physiological division is probably substantially as follows:

Time of Coitus as Recorded.	*Time of Coitus in Relation to Actual Stage in Œstrus of Individual.*
"Early."	Early + some in middle division.
"Middle."	Middle + some in early stage + an equal number in late stage.
"Late."	Late + some in middle division.

Finally there seems no reason to think that a psychological bias, conscious or unconscious, on the part of the breeders has influenced the returns. The opinions of breeders in regard to sex determination, so far as we have been able to get at them, are as follows: The majority of cattle breeders attach no significance to the time of service as a factor in influencing sex. Either they have never heard of any theory regarding the matter, or if they have are not disposed to attach any significance to it, or to modify or regulate their breeding practice in any way to conform to such a theory. A much smaller number of breeders have a decided opinion on the matter, which they usually believe to be supported in greater or less degree by their own experience. This latter class falls into two groups, about equal in numbers, and about equally dogmatic in their adherence to their particular views. One group believes that in order to secure a preponderance of females service must occur *early* in the "heat," whereas the other group maintains that to reach this end service must be *late* in the "heat." Thus one group offsets the other. Even from the men in these classes, however, the returns bear every evidence of being carefully and honestly made. An occasional somewhat rueful comment may be pencilled on the blank when the writer's theory failed to "work," but further than this preconceptions appear to have played no part.

The material would appear, from the biometrical standpoint, to be particularly favorable on the following accounts:

1. The sampling is strictly random. The different breeds are represented in substantially the proportions in which they

occurred in the State at the time of the inquiry. The statistics do not represent one or even a few large herds, but a large number of small herds scattered all over the State.

2. The original records were made by persons who knew nothing whatever of what use was to be made of them, or anything about what they "ought" to show in order to please or satisfy the person who collected them.

3. The numbers are sufficiently large to reduce the probable error of the sex ratios to reasonably small magnitudes. Of course, still more figures would be desirable, but the data as they stand are by far the most extensive yet collected on the point at issue. In the present state of knowledge regarding sex-determination it is desirable to submit to careful analysis any collection of statistics sufficiently extensive to be worthy the name.

Of course, any one considering the sex-determination problem from the statistical standpoint should be clear as to the nature of statistical evidence in general. Nothing can ever be finally "proved" by statistical evidence alone. All that any amount of statistical data can do is to state a *particular* set of facts, which are a sample, strictly localized in time and space, and restricted in magnitude, out of the whole universe of facts of like kind. If the counting has been correctly done the results from the sample are absolutely and unshakably *true, so far as concerns the sample itself.* Whether or not the sample fairly represents the relations in the whole universe to which it belongs, can never be *absolutely* determined, no matter how large the sample may be. All that can be ascertained is a more or less close approximation to the mathematical probability that the sample gives a just representation of the population or universe from which it came.

The most extensive statistics which have been published on the gross (*i. e.*, unanalyzed) sex ratio in cattle are those of Wilckens.[1] They embrace 4,900 calves and the sex distribution is 2,536 ♂ : 2,364 ♀, giving a sex ratio of 107.3 ♂ : 100 ♀.

[1] Wilckens, M., "Untersuchung über das Geschlechtsverhältniss und die Ursachen der Geschlechtsbildung bei Hausthieren," *Landwirtsch. Jahrb.*, Bd. 15, pp. 611–654, 1886.

Data.

The sex distribution of 480 calves, in relation to the time of coitus in the œstrous period, is exhibited in Table I. Besides the absolute figure the sex-ratios calculated as number of ♂♂ per 100 ♀♀ and the percentages of ♂♂ and ♀♀ and their probable errors are given.

TABLE I.

SHOWING THE NUMBER OF CALVES OF EACH SEX, AND THE SEX RATIOS, FOLLOWING COITUS AT SPECIFIED TIMES IN THE ŒSTROUS PERIOD.

Time of Coitus.	Total Offspring.	Sex of Offspring.		Percentage of		♂♂ to 100 ♀♀
		♂♂	♀♀	♂ Births.	♀ Births.	
Early in heat	248	123	125	49.60 ± 2.14	50.40	98.4
Middle of heat	125	67	58	53.60 ± 3.01	46.40	115.5
Late in heat	107	65	42	60.75 ± 3.19	39.25	154.8
Totals, all periods	480	255	225	53.13 ± 1.54	46.87	113.3

From this table the following points are to be noted:

1. The proportion of male births increases steadily with the later occurrence of coitus in the œstrous period.

2. Comparing the extremes there are more than 10 per cent. more males produced when coitus is late in heat than when it is early in the œstrous period.

3. Taking the three groups together the total figures give a sex-ratio intermediate between those exhibited in the extreme groups.

Now to consider the important question: Are the differences between the different groups shown in Table I., statistically significant, or are they such as probably arise from the inevitable "error" of random sampling? To get light on this point recourse must be had to the probable errors of the sex-proportions.

The probable errors given in the percentage column for the male births are calculated from the usual formula[1] for the probable error of a proportion or percentage, viz:

$$\text{P.E.} = .67449 \sqrt{\frac{p \cdot q}{n}},$$

[1] See G. Udny Yule's "An Introduction to the Theory of Statistics," London, 1911 (Griffin & Company), for a very clear discussion of this and other probable error formulæ.

where p denotes the proportion of "successes," q the proportion of "failures," and n the number in the sample.

Remembering that between two uncorrelated events the probable error of a difference is the square root of the sum of the squares of the probable errors of the quantities between which the difference is taken, we have the results set forth in Table II. From this table it is possible to form a first judgment as to the statistical significance of the raw data of Table I.

TABLE II.

SHOWING THE PROBABLE ERRORS OF THE DIFFERENCES BETWEEN CERTAIN OF THE SEX-RATIOS OF TABLE I.

Groups Compared.	Difference.	Probable Error of Difference.	Difference. / P.E. Diff.
Per cent. ♂ births "late in heat"—"early in heat"..	11.15	±3.84	2.90
Per cent. ♂ births "late in heat"—"middle of heat"..	7.15	±4.39	1.63
Per cent. ♂ births "middle of heat"—"early in heat"..	4.00	±3.69	1.08

From this table we note that in the case of the extreme groups the difference is 2.9 times its probable error. This means[1] that if the time of coitus in relation to œstrus were absolutely without influence on sex an excess of 11 per cent. of males such as is observed in the extreme case would only happen 5 times in every hundred that the matter was tested with statistical samples of the present size. Or, put in another way, the odds are 19 to 1 against the excess of ♂ births in "late in heat" matings being due to "chance" (*i. e.*, to errors of sampling) on the basis of probable errors here used. These are distinctly "long odds."

Can the probable errors calculated by the formula used above be relied upon to give correct results on data like the present? They assume normality of the error distributions. This assumption is practically justified in the present instance. The material may, however, be dealt with according to the method given by Pearson.[2] The present case may be regarded as falling

[1] This statement regarding probabilities assumes that the errors of sampling for this material follow substantially the normal or Gaussian curve of error See below.

[2] Pearson, K., "On the Influence of Past Experience on Future Expectation," *Phil. Mag.*, March, 1907, pp. 365–378.

in the category of estimating future expectation on the basis of experience furnished by a previous sampling. Pearson (*loc. cit.*) shows that only when a first sample is indefinitely larger than a second can we with entire accuracy take the probable error of the latter as $.67449\sqrt{mpq}$, when p and q are the chances of success and failure respectively in the former. He further shows that when a first sample n gives a percentage value of the character equal to $\bar{p}$, a second sample of m individuals may be expected to give

$$100\,\bar{p} \pm 67.449\sqrt{\bar{p}\bar{q}\left(\frac{1}{m}+\frac{1}{n}\right)} \text{ per cent.}$$

In the present instance we may take the cases of service early in heat as giving a "first sample" for the sex ratio in cattle, and then proceed to determine whether the groups served in the middle of heat and late in heat are to be regarded as random samples from the "early in heat" population.

We then have for the cases of service in middle of heat

$$\begin{aligned} \bar{p} &= .496, \\ \bar{q} &= .504, \\ m &= 125, \\ n &= 248, \end{aligned}$$

whence the expectation for the percentage of ♂ births from cows served in the middle of heat, provided time or service had *no* influence on sex, any deviation being due to errors of sampling, is 49.6 ± 3.7. Or, as a result purely of random sampling a second sample from the "early in heat" population would be expected to show as often as not a ♂ sex percentage anywhere between the limits 45.9 and 53.3.

Actually the observed percentage of males for service in "middle of heat" was 53.6, practically the same as the upper limit given.

For cases of service in last of heat we have $m = 107$, and the other values as before. Whence it is deduced, by the same reasoning as before, that the expectation for the percentage of male births from cows served *late* in the period of heat, provided time of service had *no* influence on sex, any deviation being due to errors of random sampling, would be 49.6 ± 3.9.

This means that a random sample of the size of this "late in heat" groups, from a population having the sex ratio exhibited by the "early in heat" sample, would be expected as often as not to give a male sex percentage within the limits 45.7 and 53.5.

The observed percentage of male births in the "late in heat" lot was 60.75. This value is well outside the limits, and the probability against its being a result of random sampling is considerable. It is not, however, so great as to amount to certainty. The standard deviation of the error distribution for random samples of the size of this "late in heat" lot is approximately 6.2. Taking the rough rule that for practical purposes the half range as either side of the mean is 3 σ, it would give an upper limit to the curve of 67.9. The observed 60.75, while getting well out towards the range end, still lacks something of actually reaching that point, to say nothing of going beyond.

In general, the data presented appear to warrant the following conclusions: *So far as the present statistics are concerned there is an increasing proportion of male births as the time of service approaches the termination of the œstrous period.* This increase amounts to approximately 10 per cent. in the extreme case, and to not quite half this in the intermediate case. Statistically considered the difference in the extreme case is *probably* significant (*i. e.*, not due to errors of random sampling). The probability that the observed increase in the proportions of male births is a real and biologically significant phenomenon, while not enormously great, is nevertheless of an order which one would not hesitate to act upon in the practical affairs of life.

So much for the bare facts. We have now to consider the possibilities as to the cause of the observed relation. *Apparently* the increase in male births is associated with time of service, but before accepting such a conclusion it is important to determine whether some other variable factor may not be influencing the results. The first possibility which suggests itself is that there may be a difference in age distribution of the animals in the three lots and that in some manner this has affected the result. In the ideal experiment of this sort of course the parents used should be of the same average age in the different groups.

The age distribution of the animals involved in these statistical

returns has been examined with the results shown in Tables III. and IV. Table III. gives the actual frequency distribution, and Table IV. the constants calculated therefrom. It should be said that in these distributions the parents are weighted with their fertility. That is, each individual appears once for each offspring. This seems the fairest manner in which to deal with the problem, for comparisons such as are here indicated.

TABLE III.

SHOWING THE AGE DISTRIBUTION OF THE ANIMALS IN THE SEVERAL GROUPS.

Age in Years.	Service Early in Heat.		Service Middle of Heat.		Service Late in Heat.	
	No. of ♂♂.	No. of ♀♀.	No. of ♂♂.	No. of ♀♀.	No. of ♂♂.	No. of ♀♀.
1– 1.9	71	7	44	4	25	2
2– 2.9	68	26	26	15	49	12
3– 3.9	39	31	16	11	13	15
4– 4.9	8	23	4	13	2	13
5– 5.9	17	16	5	12	1	8
6– 6.9	2	30	1	10	2	12
7– 7.9	—	27	—	7	—	12
8– 8.9	—	13	—	10	—	11
9– 9.9	—	8	—	6	—	8
10–10.9	—	16	—	6	—	5
11–11.9	—	7	—	2	—	2
12–12.9	—	4	—	6	—	1
13–13.9	—	2	—	—	—	1
14–14.9	—	0	—	—	—	—
15–15.9	—	1	—	—	—	—
Totals[1]	205	211	96	102	92	102

From these distributions the constants of Table IV. have been calculated by the ordinary biometric methods.

TABLE IV.

SHOWING THE VARIATION CONSTANTS FOR THE AGE OF BREEDING CATTLE.

Group and Sex.	Mean, Years.	Standard Deviation, Years.	Coefficient of Variation, Per Cent.
Service early in heat—♂♂	2.71 ± .06	1.21 ± .04	44.7
Service middle of heat—♂♂	2.49 ± .08	1.16 ± .06	46.6
Service late in heat—♂♂	2.53 ± .07	.93 ± .05	36.6
Service early in heat—♀♀	6.16 ± .14	2.95 ± .10	47.9
Service middle of heat—♀♀	6.15 ± .20	3.04 ± .14	49.5
Service late in heat—♀♀	6.15 ± .18	2.73 ± .13	44.4

[1] The differences between these and the offspring totals are due in the main to failures in individual cases to record the age of an animal in the schedules. In small part they are due to multiple gestation.

Obviously there are no differences in age distribution of sufficient magnitude to cause the observed differences in sex-ratios among the three groups. The males used in the three groups are of very nearly the same mean age. The same is true to an even more marked degree of the females of the three groups. It would be difficult to collect a more homogeneous lot of statistics than the present one, so far as concerns age distribution of the individuals in the several samples.

We have not been able to discover any other factor which could account for the observed differences in the sex-ratios described above. By the most careful tests which we have been able to apply the material appears to be statistically homogeneous except in respect to the time of service.

Some of the individual records are of interest. The following schedule from Mr. C. E. Clifford may be cited in particular. He was a breeder of registered Jersey cattle and carefully followed a regular system of service in relation to time of heat. This method in his own words was as follows: "Cover cow twice *immediately* upon disccvery of heat and keep her separate from all [other cattle] until heat is entirely passed." The sex distribution for 14 calves born in the year for which the return was made was 2 ♂♂ and 12 ♀♀.

Discussion.

It will have been noted that the results set forth above support, in essential features, the theory of Thury (*loc. cit.*) as to sex determination in cattle. Thury's original paper was published in French under the title "Memoir sur la loi de production des sexes chez les plantes, les animaux et l'homme." It immediately attracted the attention of biologists and was critically reviewed and discussed in the *Zeitschrift für wissenschaftliche Zoologie* (Bd. XIII., Heft 4) by Pagenstecher. He later translated Thury's paper into German, added appendices, etc., and it is this German translation which has chiefly been cited by later workers. The essential features of Thury's theory, so far as concerns mammals normally bearing a single young at birth, were set forth in the following words (Thury, *loc. cit.*, p. 16).

"Das Geschlecht hängt ab vom Grade der Reifung des Eies im Augenblicke, wo es von der Befruchtung getroffen wird.

"Das Ei, welches, wenn es befruchtet wird, noch nicht einen gewissen Grad der Reifung erreicht hat, giebt ein Weibchen; ist dieser Grad der Reifung überschritten, so giebt das Ei, wenn es befruchtet wird, ein Männchen.

"Wenn zur Zeit der Brunst, ein einziges Ei, vom Eierstock abgelöst, langsam durch den Geschlechtsapparat herabsteigt (Thiere, welche ein Junges gebären), so genügt, es dass die Befruchtung am Anfange der Brunst statthabe, um Weibchen zu zeugen, und am Ende, um Männchen zu zeugen, indem die Umwandlung (vire) des Zustandes des Eies normal während der Dauer seines Durchganges durch den Geschlechtskanal stattfindet."

Curiously enough this theory, while exciting much interest at the time of its appearance, has never been given any adequate experimental-statistical test. The only experimental evidence which Thury himself offered in its favor was that obtained by a friend Cornaz[1] which was so brilliantly confirmatory as to arouse suspicion in the minds of all subsequent workers, with the result that it has never been regarded as of any particular worth. At the best it was ridiculously inadequate statistically. Cornaz states that by following Thury's directions as to time of service he obtained 22 female calves in unbroken succession. In 7 cases he desired and obtained male calves, the service in these cases being late in the heat.

Düsing (*loc. cit.*) discussed Thury's theory at great length, and pointed out in the following words one of its obviously great weaknesses (p. 16): "Indessen ist diese eigentliche Thury-sche Theorie in sofern nicht richtig, als immer nur ein gewisser Überschuss des einen oder andern Geschlechtes erwartet werden kann." Thury postulated *complete* determination.

Later writers have dismissed Thury's theory with rather scant consideration. Thus F. H. A. Marshall (*loc. cit.*, p. 630) says: "Thury claimed that he could regulate the sexes in cattle by allowing coitus only at the beginning or at the end of œstrous periods, . . . but other investigators have failed to establish Thury's conclusions." Morgan[2] (p. 393) says: "Thury based

[1] Published as a part of Thury's paper (*loc. cit.*), pp. 17 and 18.

[2] Morgan, T. H., "Experimental Zoölogy," New York, 1907.

based his conclusions on 29 experiments with cows, in which union took place at the beginning or at the end of the period of heat. Others have failed to confirm his conclusions, and contradictory results have been obtained with rabbits, and hens." Neither Marshall nor Morgan give any detailed references to the literature.

F. R. Marshall[1] evidently has Thury's theory in mind in the following statement but has apparently received the tradition in slightly garbled form: "The commonest idea about sex determination is that females bred at the beginning of the period of heat produce male offspring. Other notions are based on the same supposed principle, namely, that an ovum fertilized while immature produces a male; maturity is supposed to be in proportion to the age of the ovum and the nutritive condition of the dam." He then proceeds (on grounds it must be said which are strictly *a priori*) to demolish all such notions.

So far as we have been able to discover the following are the only experimental tests which have ever been accorded Thury's theory as regards cattle breeding, which at all adequately fulfill the conditions necessary for a critical test. Supposed tests of the theory carried out with plants, multiparous mammals, birds, etc., will not be discussed here, because it seems to us either (*a*) obvious *a priori* that the theory could not possibly apply, in the nature of the case, to some of the organisms and forms of reproduction which Thury and subsequent workers attempted to include in its purview, or (*b*) that the so-called tests of the theory were in many instances absolutely uncritical and could not have been expected under the most favorable circumstances to have yielded any really significant results one way or the other. The tests with cattle, which if carefully carried out must yield critical evidence, are, as has been said, meager enough in number and magnitude. The first were made shortly after the publication of the theory at the agricultural academies at Proskau and Eldena.[2] The results were as follows:

[1] Marshall, F. R., "Breeding Farm Animals," Chicago, 1911.

[2] *Annalen der Landwirtschaft*, Jahrg. 23, Bd. 46, p. 271, 1865. For the facts regarding these and the following tests we are indebted to Düsing's (*loc. cit.*) memoir.

Time of Service.	Sex of Young. ♂	♀
Early in heat	8	10
Late in heat	4	1

From these figures it was concluded that the theory in the bald form proposed by Thury was untenable.

The matter was again tested in Waldau and Eldena[1] with the following results:

Time of Service.	Sex of Young. ♂	♀
Early in heat	3	10
Late in heat	1	1

The last experimental test of the matter of which we have been able to find any report (except that of Russell, *loc. cit.*) is that of Touchon[2] who obtained the following results.

Time of Service.	Sex of Young. ♂	♀
Early in heat	0	11

These figures are very far from extensive. In view of the great practical importance of the matter, however, one would have thought that they would have acted as a stimulus to more adequate researches.

It is worth while to put together all the data regarding the relation of time of service to sex in cattle. We have the following figures:

Time of Service.	Sex of Young. ♂	♀	♂♂ : 100 ♀♀
Early in heat	134	178	75.3
Middle of heat	67	58	115.5
Late in heat	77	44	175.0
Totals	278	280	

These figures are suggestive. While they certainly demonstrate that Thury's theory in its original form is untenable, on the other hand, they strongly indicate, though they admittedly do not absolutely *prove*, that the sex ratio in cattle may be modified by controlling the time of service in relation to the œstrous period. We would call attention to the fact that the

[1] *Annalen ner Landwirtschaft*, Wachenblatt, 1866, p. 461.

[2] *Agronomische Zeitung*, 1865, p. 519.

totals give approximately a 1 : 1 ratio, as on *a priori* grounds they should.

Granting the fact that the sex ratio in cattle does change as the time of service changes, what is the biological basis of the phenomenon? Thury's idea was that the sex of the offspring was determined by the relative staleness of the ova. In making this hypothesis it may be assumed either that ovulation occurs early in œstrus entirely, or as a more or less regular process throughout the œstrous period. Unfortunately no exact and thorough studies have been made on the physiology of reproduction in the cow. As has been pointed out by Marshall (*loc. cit.*, p. 316) the fact that artificial insemination has been successfully practiced with cows would indicate that ovulation is independent of coition. In a later paragraph that author states that: "There can be little doubt that in the great majority of mammals ovulation, as a general rule, occurs regularly during œstrus." This would certainly seem to be the case in the cow, since our statistics show that pregnancy may follow coitus at any time during the œstrous period. It has lately been contended by Jentsch,[1] on rather meager evidence, that cows even when served out of the œstrous period will in some cases become pregnant. The duration of life of the unfertilized egg in the uterus is not known for the cow. It has recently been shown by Lewis[2] to be distinctly brief in the case of the sow. He states as the result of numerous observations and experiments that the ovum "does not retain its vitality for more than a few hours after being liberated from the Graafian follicle." But if in the cow ovulation begins at or very near the beginning of œstrus, as seems certain from the returns in some of our schedules, where pregnancy followed a coitus within one or two hours of the beginning of œstrus, then it can fairly be considered as probable that the average age of fertilized ova following coitus late in heat will be somewhat greater than that of the ova fertilized early in heat. Nothing more than an average difference can be claimed (*i. e.*, not every individual ovum fertilized late in heat will be older

[1] Jentsch, A., "Ueber Befruchtung ausserhalb der Brunstzeit beim Rind," *Jahrb. wiss. prakt. Tierzucht.*, Bd. 6, pp. 441–444, 1911.

[2] Lewis, L. L., "The Vitality of Reproductive Cells," Oklahoma Agr. Expt. Sta. Bulletin 96, pp. 1–47, 1911.

than every individual fertilized early) and it is not possible to make any accurate estimate of what the difference amounts to. However, until some more definitely related factor is found it may be tentatively concluded, purely as a working hypothesis, that the observed changes in the sex ratios are correlated with changes in the relative freshness (or staleness) of the ova at the time of fertilization.

The hypothesis that the metabolic condition of the ovum when fertilized may influence the sex of the offspring finds support in the recent work from Richard Hertwig's laboratory. Hertwig[1] in a series of studies on frogs found that over-ripeness of the eggs was associated with a preponderance of males, extending in favorable cases to the production of 100 per cent. males. These results were criticized on several grounds. The experiments, however, have been very carefully repeated and extended by a student of Hertwig's, Kuschakewitsch,[2] who obtained similar results. The experiments were criticized by Morgan first on the ground of differential mortality, and second on the ground of differential fertilization, but Kuschakewitsch was able to show that the results were not open to criticism on these grounds. Whatever the explanation the *facts* brought out by Hertwig and Kuschakewitsch must certainly be accepted. The procedure which they followed led to a great preponderance of males. Miss King[3] has also shown that it is possible to modify significantly the sex-ratio by changing the metabolic condition of the eggs.

In the last few years a considerable body of evidence has accumulated showing that sex determination is primarily a matter of inheritance. It is not necessary to review the voluminous literature here. Essentially two points have been demon-

[1] Hertwig, R., "Ueber das Problem des sexuellen Differenzierung," *Verhandl. deutsch. Zool. Ges.*, 1905; "Weitere Untersuchungen über das Sexualitätsproblem," *ibid.*, 1906; "Weitere Untersuchungen," etc., *ibid.*, 1907.

[2] Kuschakewitsch, S., "Die Entwicklungsgeschichte der Keimdrüsen von *Rana esculenta*. Ein Beitrag zum Sexualitätsproblem," *Festschrift f. Hertwig.*, Bd. 2, 1910.

[3] King, Helen Dean, "Studies on Sex Determination in Amphibians, IV. The Effects of External Factors, Acting Before or During the Time of Fertilization, on the Sex Ratio of *Bufo lentiginosus*," BIOL. BULLETIN, Vol. XX., pp. 205–234, 1911. See also others paper in the same series.

strated. On the one hand the cytologists have shown for many forms that certain chromosomes are definitely associated with sex differentiation. On the other hand, the geneticists have shown that in a considerable number of forms the phenomenon of sex-linked inheritance is exhibited, a phenomenon which has as yet received no more cogent explanation than the obvious one that sex differentiation depends upon germinal factors, and that these behave in inheritance in accordance with Mendelian principles.

No one could rate higher the evidence that sex is determined primarily by inheritance than does the writer. But it is idle to deny that there is also a large and increasing body of critical evidence indicating that, in one way or another, sex ratios may be modified experimentally, and to some degree indeed controlled. Any adequate hypothesis of sex-determination must account both for the facts indicating innate pre-determination in the germ cells before fertilization, and also those indicating the modifying influence of external factors. Because one of these sets of facts is true does not mean that the other set is necessarily false, and should be forthwith annihilated (if possible) by destructive criticism. Such definite facts cannot be mutually exclusive. It would seem that a logical view of the case is that while sex is, in many cases at least, *primarily* determined by innate hereditary causes, nevertheless external factors, acting at the appropriate time, may in some instances modify the effect of the innate factors. This is essentially the same conclusion as is reached by Schleip[1] in his recent extensive and critical review of the literature in this field.

It is not difficult to conceive how such results could be brought about physiologically. The evidence indicates that particular chromosomes are concerned with the hereditary determination of sex. Furthermore a broad survey of the experimental work in the modification of sex-ratios by external conditions indicates that the most effective agencies in bringing about such modifications are those which directly affect the metabolic condition of the germ cells (*e. g.*, staleness, extraction of water, etc.). Why

[1] Schleip, W., "Geschlechtsbestimmende Ursachen im Tierreich," *Ergeb. u. Fortschr. Zool.*, Bd. III., pp. 165–328, 1912.

may not the sex chromosomes be affected by these agencies along with the rest of the germ cell to a sufficient degree to modify their specific effect? As has recently been pointed out:[1] "There are two ways of looking at the relationship between sex chromosomes and primary and secondary sexual characters on the assumption already made that the differential factor is essentially quantitative in nature. On the one hand it may be assumed, as it has been in most discussions of the subject, that the *X*-chromatin acts in a *positive* way, if at all, in the determination of sex. On this view two 'doses' of *X*-chromatin, in some manner not fully understood, positively determine the development of female characteristics. It has been argued that since the female is primarily anabolic in tendency, as pointed out many years ago by Geddes and Thomson, the determination of the female sex by a 'plus' condition in respect of chromatin may be explained on the assumption that *X*-chromatin is a sort of 'tropho-chromatin' in which reside the energy potentialities of the organism.

"There are two difficulties which confront this interpretation. The first is that the female characters cannot be regarded as an extension or intensification of those of the male. Rather the contrary is true. The male almost universally represents a higher degree of specialization and differentiation in development than the female. Another difficulty is found in the phenomenon of hermaphroditism.

"I would suggest that both of these difficulties may be overcome and all of the facts be better interpreted by assuming that *X*-chromatin is not a positive cause of sex differentiation but rather is an *inhibitor* of the development of male sex characters. It is a well-known fact that in the vertebrates, where the embryology of the genital organs have been most carefully studied, the male condition represents a more extended and advanced degree or stage of development than the female. The system is in every part homologous in male and female, but the latter appears objectively to have been arrested in a development which, without such arrest, would have led to the same result

[1] Pearl, R., in as yet unpublished lectures on the "Biology of Sex," delivered before the Graduate School of Agriculture, Lansing, Michigan, July 1–6, 1912.

as is seen in the male. The same consideration applies to the male and female germ cells. The ovum is much less widely differentiated from the primordial indifferent germ cells than is the spermatozoön. Now if it be assumed that two 'doses' of this specific *X*-chromatin serve to inhibit completely the development of 'maleness,' while one 'dose' is insufficient to do this, but allows the male characters to develop, we shall have, it seems to me, a more satisfactory interpretation than is gained by looking at the matter in the reverse way.

"This viewpoint would explain why it is that castrated males do not, save possibly in exceptional circumstances, take on female characters. On the other hand, according to Goodale's observations, castrated females do take on male characters. Further an explanation is found for the numerous cases in which a female in old age or after disease takes on male characters.

"On this view hermaphroditism becomes analogous to the phenomenon of the retention of larval characters in development it being assumed that there is one 'dose' or less of *X*-chromatin in such cases."

On either of these hypotheses as to the sex-determining action of the *X*-chromosomes it is not difficult to conceive how any change in the general metabolic condition of the germ cells might modify the sex ratio. The *X*-chromatin would presumably be affected along with the rest of the cell and the relative potency of its sex-determining factors changed.

In conclusion we wish our position in regard to the results set forth in this paper to be clearly understood. It is not contended or supposed by the writers that the time of service in relation to the period of heat *absolutely controls* the sex of the subsequent offspring. It is believed, however, that the facts set forth show, with a considerable degree of probability, that the sex ratio in cattle can be to some extent *modified* by controlling the time of service. But the amount of such observed modification is not so great that the matter can be tested with a few individuals. There is every reason to believe that any effect would only appear in fairly comprehensive statistics. The matter is one of much practical consequence to the stock breeder. Because this is so we would caution the reader against misinter-

preting the results of this paper. A trial on a half dozen individuals will not in any sense whatever adequately test the accuracy of the results set forth in this paper. Nor will a breeding experiment with any other animal than the cow. If any person chooses to generalize from the data of this paper that in animals in general, or indeed in any other animal than the cow, time of service and sex of offspring are causally related, the responsibility for such generalization must be *entirely* his, not ours. We have studied statistics for cows in regard to this problem, and as yet no others, and have tried to make clear just what these data show. Beyond this solid ground of fact we do not care to venture, particularly so far as concerns the practical application of these results by the breeder.

Time of service is very evidently no absolute determining factor for sex in cattle. On the other hand the probability that the sex ratio can be changed by careful attention to this matter of time of service is sufficiently great, in our judgment, to warrant any man in modifying his breeding practice in accordance with it, particularly since in so doing he will be incurring no added risk of any kind. In the every-day affairs of life in regard to business, investment of funds, and the like, practical men every day undertake courses of action on the basis of probabilities much smaller than that in favor of getting an increased number of males if cows are served late in the heat. The practical cattle breeder in most cases would like, if he could get it, an excess of female calves. All the evidence at hand warrants the belief that by taking care that cows are served *as soon as possible* after the onset of heat there will be some reduction in the proportion of male calves born. In short, the facts set forth in this paper warrant the breeder in paying attention to the time of service in his cattle breeding operations, but he must not suppose that by so doing he can absolutely control the sex of the offspring, or even approach measurably close to absolute control. He can at best merely modify, over a period of years, the sex ratio in greater or less degree, in the direction which he desires.

SUMMARY.

In this paper statistics collected some years ago at the Maine Agricultural Experiment Station in regard to the relation between

time of service in the œstrous period and the sex of the subsequent offspring in domestic cattle are subjected to biometric analysis. These statistics are much more extensive than any which have hitherto been collected for the study of this problem in cattle. It is shown:

1. That as the time of coitus approaches the end of the œstrous period there is a progressive increase in the proportion of male young born.

2. That in the extreme case this increase in the proportion of male births is probably statistically significant and not to be attributed to errors of random sampling.

3. That these modifications of the sex ratio cannot be attributed to age differences or to any other factor yet suggested.

A possible explanation of the results and their practical bearings are discussed.